Transcriptional profiling *of Pseudomonas aeruginosa* mature single and dual species biofilms in response to meropenem

Farhana Alam[1], Jessica M. A. Blair[2], Rebecca A Hall[3#]

[1]Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, B15 2TT

[2]Institute of Microbiology and Infection, College of Medical and Dental Sciences, University of Birmingham, B15 2TT

[3]Kent Fungal Group, Division of Natural Sciences, School of Biosciences, University of Kent CT2 7NJ

corresponding author r.a.hall@kent.ac.uk

Key words: *Candida albicans*, *Pseudomonas aeruginosa*, meropenem, transcriptional profiling, dual species biofilms

Repositories: Sequencing reads and gene hit count tables are available at the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/), under accession number GSE167137.

Abstract

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen frequently isolated from chronic infections of the Cystic Fibrosis lung and burn wounds, and is a major cause of antimicrobial resistant nosocomial infections. *P. aeruginosa* is frequently co-isolated with the opportunistic fungal pathogen *C. albicans,* with the presence of *C. albicans* in dual species biofilms promoting tolerance to meropenem. Here, transcription profiling of mature *P. aeruginosa* single or dual species biofilms was carried out to understand the molecular mechanism(s) by which *C. albicans* enhances meropenem tolerance. *C. albicans* appeared to have a mild impact on the transcriptome of *P. aeruginosa* mature biofilms, with most differentially regulated genes being involved in interkingdom interactions (i.e. quorum sensing, and phenazine biosynthesis). The addition of meropenem to mature single or dual species biofilms resulted in a significant bacterial transcriptional response, including the induction of the beta-lactamase, *ampC*, genes involved in biofilm formation. *P. aeruginosa*, elicited a similar transcriptional response to meropenem in the presence of *C. albicans*, but *C. albicans* promoted the expression of additional efflux pumps, which could play roles in increasing the tolerance of *P. aeruginosa* to meropenem.

Introduction

Pseudomonas aeruginosa is a Gram-negative bacterial pathogen associated with chronic infections in the Cystic Fibrosis (CF) lung (Lyczak *et al.* 2002), burn wounds (Ramakrishnan *et al.* 2016) and is a major contributor to nosocomial infections (Chatterjee *et al.* 2016). The ability of the bacterium to form biofilms is critical to the pathogenicity of *P. aeruginosa*, with biofilms being the predominant mode of growth in both the CF lung and wounds (Bjarnsholt *et al.* 2009, Fazli *et al.* 2009). Biofilms are of medical importance as they protect the microbes from the host's innate immune system (Alhede *et al.* 2009, Bjarnsholt *et al.* 2009), and significantly enhance antimicrobial resistance, with biofilms being 100-1000 times more resistant to antimicrobial therapies than their planktonic counterparts (Mah and O'Toole 2001). Furthermore, cells dispersed from biofilms exhibit a unique transcription profile (Guilhen *et al.* 2016), suggesting that dispersed cells represent a distinct stage which may enhance dissemination and infection progression.

P. aeruginosa is frequently co-isolated from sites of infection with the fungal pathogen *Candida albicans* (Doern and Brogden-Torres 1992). These two microbes undergo complex interactions including direct cell-cell interactions, and through the secretion of signalling molecules and metabolites (Fourie and Pohl 2019), the outcome of which is dependent on environmental factors. During biofilm formation, *P. aeruginosa* use the fungal hyphae as a scaffold to enhance the structure, composition and complexity of the biofilm (Hogan and Kolter 2002, Brand *et al.* 2008). The presence of *C. albicans* in biofilms enhances antibiotic tolerance of several important bacterial pathogens, including *P. aeruginosa* (Harriott and Noverr 2009, Kong *et al.* 2016, Alam *et al.* 2020). Enhanced antimicrobial resistance is hypothesised to result from increased extracellular matrix production in dual species biofilms, as a result of the fungus contributing cell wall carbohydrates like glucans and mannans to the matrix (Harriott and Noverr 2009, De Brucker *et al.* 2015, Kong *et al.* 2016, Alam *et al.* 2020). These fungal polysaccharides are thought to provide protection against antimicrobial agents through either limiting the diffusion of the antimicrobial through the biofilm (i.e. vancomycin) (Kong *et al.* 2016), or by binding and sequestering the drugs (i.e. azoles) (Nett *et al.* 2007, Mitchell *et al.* 2015).

Meropenem is a carbapenem antibiotic that is frequently used to treat chronic *P. aeruginosa* infections. Previously we have shown that in dual species biofilms *C. albicans* increases the tolerance of *P. aeruginosa* to meropenem, a process dependent on fungal mannan (Alam *et al.* 2020). To provide more detail on the molecular mechanism(s) underlying this phenomenon, we analysed the transcriptional response of mature *P. aeruginosa* biofilms, and dual species biofilms in the absence and presence of meropenem. The transcriptional profile identifies key

P. aeruginosa genes and biological processes required for resistance to meropenem. Similar genes and processes were unregulated by *P. aeruginosa* in dual species biofilms indicating that the presence of *C. albicans* in mature biofilms does not perturb the *P. aeruginosa* transcriptional response to meropenem.

Methods

Strains and Media

C. albicans SC5314 was grown and maintained on yeast peptone dextrose (YPD, Sigma) media, while *P. aeruginosa* PAO1 (ATCC15692) was grown and maintained on LB medium. All biofilm assays were performed in Muller-Hinton broth (MHB).

Biofilm assay

Biofilm assays were based on previously described methodology (Alam *et al.* 2020), but scaled up to 6-well plates. In brief, overnight cultures of *C. albicans* and *P. aeruginosa* were washed in PBS, and *C. albicans* resuspended at 1 x10^6 cells/ml and *P. aeruginosa* to OD600 of 0.2 (~2 x10^8 CFU/ml) in Mueller-Hinton broth. Each well contained 3 ml *C. albicans* and 300 μl of *P. aeruginosa* in a total of 6 ml. Plates were incubated at 37ºC for 2 hr to allow cells to adhere, at which point the media was replaced with fresh sterile media, and plates incubated statically at 37ºC for 24 hrs. Cells not part of the biofilm were removed and media replaced with fresh MHB containing 0 or 5 μg/ml meropenem, and plates incubated for 4 hrs. Media was replaced with 2 ml PBS containing 50 μg/ml DNase I and plates incubated at 37ºC for 1 hr to degrade the extracellular matrix. Biofilms were detached from the plate by scraping, serially diluted, and plated onto selective agar (YPD agar supplemented with 100 μg/ml tetracycline to determine viable *C. albicans* CFUs and Cetrimide agar to determine viable *P. aeruginosa* CFUs).

Preparation of samples for RNA extraction

Biofilms were formed as described above and triplicate biofilms were pool and 50 μl serially diluted and plated on cetrimide agar or YPD supplemented with 100 μg/ml tetracycline to check for contamination. Remaining biofilm cells were centrifuged at 3500 rpm at 4ºC for 5 min and pellets snap frozen in liquid nitrogen. Four biological replicates were shipped to GeneWiz®, UK, for RNA extraction sequencing and basic bioinformatic analysis.

RNA extraction and sequencing

RNA was extracted from the biofilms by GeneWiz® using the Qiagen RNeasy Plus mini kit. Library preparation was done in the following stages: A) ribosomal RNA depletion; B) RNA

fragmentation and random priming; C) first and second strand cDNA synthesis; D) end repair, 5' phosphorylation and dA-tailing; E) adapter ligation, PCR enrichment and sequencing. Paired-end sequencing was performed using Illumina HiSeq 4000 (2x150bp configuration, single index, per lane).

Bioinformatic analysis

Sequence quality of each sample was evaluated by determination of the number of reads, the yield (Mbases), the mean quality score, and the percentage of reads over 30 bases in length. FastQC software was used to determine per base sequence quality and per sequence GC content. Sequence reads were trimmed to remove adapter sequences and nucleotides with poor quality, using Trimmomatic v.0.36. The trimmed reads were mapped to the *P. aeruginosa* reference genome, available on ENSEMBL, using the STAR aligner v.2.5.2b. For dual species biofilms, samples were treated the same as single species biofilms. Reads were mapped to the PAO1 genome, and non-mapped reads were discarded and later aligned to the *C. albicans* reference genome. Unique gene hit counts were calculated using featureCounts from the Subread package v.1.5.2. Only unique reads that fell within exonic regions were counted (for unique genes, the number of hits per read was set to 10 as default, with reads that mapped to less than 10 distinct places be assigned to one place by the EM algorithm, and reads that mapped to more than 10 distinct places being discarded). For the analysis, only read counts for genes in the *P. aeruginosa* genome were used and the TPM values calculated as follows: each read count was divided by the length of each gene in Kb to generate reads per kb (RPK), then all the RPK values in the sample were counted and divided by 1,000,000 to generate the scaling factor, and finally the RPK values were divided by the scaling factor to generate the TPM value. Differential gene expression analysis was performed using DESeq2 and the comparisons listed in Table 1. Principal component analysis (PCA) was performed to reveal the similarities within and between groups, with PCA plots included in the output (Figure S1). As expected, dual species biofilms exhibited greater biological variation than single species biofilms (Figure S1), which likely reflects the heterogeneity of the biofilm structure. Meropenem treatment had the greatest effect on the bacteria transcriptome, with samples clustering into distinct groups in the PCA plots, while *C. albicans* had a reduced impact on the *P. aeruginosa* transcriptome, resulting in great spread of the data. DESeq2 output files for each comparison, and files containing summary raw and normalised reads and TPM values for each gene are available at the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/), under accession number GSE167137.

Enrichment analysis

For *P. aeruginosa* transcriptomic analysis, differential expression of genes between conditions was considered significant if the adjusted P-value (Padj) was ≤0.05. Gene ontology (GO) analysis was done using KOBAS 3.0 software (KEGG (Kyoto Encyclopaedia of Genes and Genomes) Orthology Based Annotation System) (Xie *et al.* 2011).

Statistical analysis

Biofilm data were analysed in GraphPad Prism (version 9.1.0) using 2-way ANOVA and Holm-Sidak's multiple comparisons test.

Results

C. albicans enhances the tolerance of *P. aeruginosa* biofilms to meropenem even at early timepoints.

Previously we have observed that when *P. aeruginosa* is grown in a dual species biofilm with the fungal pathogen *C. albicans*, the tolerance of *P. aeruginosa* to meropenem is increased (Alam *et al.* 2020). To understand the molecular mechanism(s) behind this increased tolerance transcriptional profiling was performed. To avoid the transcriptional profile focusing on genes related to cell death, we analysed the transcriptome after the antibiotic had been added to mature biofilms for 4 hours. Plating of mono and dual species biofilms in the absence and presence of meropenem confirmed that the majority of the cell population was viable at this time point. Furthermore, the tolerance of *P. aeruginosa* to meropenem in the presence of *C. albicans* was still enhanced even at this early timepoint (Figure 1).

Meropenem enhances *ampC* expression in *P. aeruginosa* single species biofilms

Addition of meropenem to *P. aeruginosa* mono species biofilms resulted in the significant upregulation of 354 genes, while 509 genes were downregulated (Figure 2, Figure S2A, Table 2). Of the significantly differentially regulated genes 45% (159/354) and 43% (217/509) encoded hypothetical proteins. As expected, the most significantly up regulated gene in response to meropenem treatment was *ampC* ($\log_2$ fold change = 7.75, Padj = $1.95\ 10^{-42}$), which encodes a beta-lactamase, important for carbapenem resistance. GO term enrichment analysis of the differentially regulated genes confirmed that meropenem resulted in the significant upregulation of genes involved in the maintenance of the bacterial cell membrane, cell wall, biofilm formation and extracellular matrix production, siderophore production, type IV pilus formation, and type VI secretion system, while genes involved in putrescine transport were significantly downregulated (Figure 3A). KEGG pathway analysis, identified the over representation of genes involved in siderophore biosynthesis, vancomycin resistance, amino acid metabolism, biofilm formation and bacterial secretion in genes that were significantly up

regulated and pathways associated with thiamine metabolism, mismatch repair, pyrimidine metabolism, and purine metabolism were significantly down regulated (Figure 3B).

The presence of *C. albicans* in dual species biofilms results in mild changes in the *P. aeruginosa* transcriptome.

To determine how the presence of *C. albicans* affected *P. aeruginosa*, we compared the transcriptional profile of *P. aeruginosa* single species biofilms to dual species biofilms. The presence of *C. albicans* resulted in the differential regulation of 392 genes, with 265 genes being significantly upregulated and 127 genes being significantly down regulated (Figure 2, Figure S2B, Table 3). Although significant, we note that the $\log_2$ values for the majority of these genes was less than 1, suggesting that *C. albicans* has a mild impact on the transcription profile of *P. aeruginosa*. The most significantly differentially regulated genes were PA4097 (a lipolytic protein) and PA5384 (an alcohol dehydrogenase) which were both 2-fold upregulated in the presence of *C. albicans*. GO term enrichment analysis (Figure 4A), and KEGG pathway analysis (Figure 4B) of the 392 differentially regulated genes identified that phenazine biosynthesis, *Pseudomonas* quinolone signal (PQS) production, pyridocal phosphate biosynthesis, and siderophore transport and pyoverdine biosynthesis were significantly upregulated while genes involved in type IV pilus biogenesis, the general secretory (Sec) pathway, and amino acid biosynthesis were significantly down regulated. Therefore, *P. aeruginosa* appears to upregulate processes that might provide the bacterium with a competitive advantage when growing in the presence of *C. albicans.*

To identify whether the presence of the fungus was inducing a similar transcriptional response to the drug treatment we compared DEGs identified in *P. aeruginosa* single species biofilms treated with meropenem to the list of DEGs identified in untreated dual species biofilms. Seven genes (PA10907, PA4830, PA4518, PA1209, *rimK*, PA0881 and PA3758) were upregulated, while five genes (*pyrD*, *aroC*, PA4633, PA3526 and PA4637) were down regulated under both conditions. However, as the majority of the genes encode hypothetical proteins the impact of these transcriptional responses is unknown.

Impact of *C. albicans* on the *P. aeruginosa* transcriptional response to meropenem

To determine whether the presence of *C. albicans* affected the response of *P. aeruginosa* to meropenem, we compared the transcriptional profile of untreated dual species biofilms to meropenem treated dual species biofilms. In response to meropenem, a total of 620 genes were differentially regulated, with 304 genes being significantly upregulated, and 316 being significantly downregulated (Figure 2, Figure S2C, Table 4). As was the case for the single species biofilms, *ampC* was the most significantly upregulated gene ($\log_2$ = 5.52, Padj = 1.95

10^{-47}). GO term enrichment analysis (Figure 5A) and KEGG pathway analysis (Figure 5B) confirmed that like single species biofilms, treatment of *P. aeruginosa* dual species biofilms with meropenem resulted in the upregulation of genes involved in cell division, peptidoglycan biosynthesis, biofilm formation, extracellular matrix production siderophore biosynthesis and type VI secretion. Likewise, the processes that were significantly downregulated in single species biofilms in response to meropenem (i.e. putrescine transport and mismatch repair) where shared with dual species biofilms (Figure 5).

Although *C. albicans* does not affect the general processes that are differentially regulated in response to meropenem, it is possible that the underlying DEGs are different. Therefore, we compared the DEGs between single and dual species biofilms in response to meropenem. Of the genes that were significantly upregulated in response to meropenem, 181 (37.9%) were upregulated in both single and dual species biofilms, while 173 (36.3%) and 123 (25.8%) were unique to single, and dual species biofilms, respectively (Figure 6A). Among the upregulated genes that were unique to the dual-species meropenem response, there were three linked to outer membrane vesicles (*pagL*, *galU* and PA5441) (Choi *et al.* 2011), one involved in cell wall synthesis (*ddlA*), three involved in biofilm formation (*pslH*, *pslI* and *algR*), and three involved in efflux (PA1809, *mexC* and PA3314). This suggests that the presence of *C. albicans* may increase the ability of *P. aeruginosa* to form robust biofilms, to secrete molecules via outer membrane vesicles (OMVs) and the MexCD-OprJ efflux pump, which may play a role in increasing the tolerance of the bacterium to meropenem.

Of the genes that were significantly downregulated in response to meropenem, 282 (51.9%) genes were shared in common between single and dual species *P. aeruginosa* biofilm cells, while 227 (41.8%) and 34 (6.3%) were unique to single and dual species biofilms respectively, (Figure 6B). Among the few downregulated genes that were unique to the dual-species meropenem response, there were six involved in efflux or membrane transport (PA0603, PA0604, *mexE*, *mexF*, PA0860 and PA1051). This suggests that the presence of *C. albicans* may result in reduced production of the MexEF-OprN efflux pump in *P. aeruginosa* biofilm cells.

Discussion

Biofilms are medically important as they result in antimicrobial resistance and protect microbes from the actions of the immune system, making infection harder to treat. Furthermore, biofilms are normally mixed species communities, with our understanding of how microbe-microbe interactions within these communities affect disease progression being limited. We have previously shown that *C. albicans* enhances the tolerance of *P. aeruginosa* to meropenem, a

commonly used carbapenem for the treatment of chronic *P. aeruginosa* infections. Therefore, transcriptional analysis was carried out to understand how *C. albicans* promotes meropenem tolerance.

Mechanisms of resistance to the carbapenem class of antibiotics include decreased outer membrane permeability, beta-lactamase expression, increased efflux and target modification. In agreement with this, the addition of meropenem to mature *P. aeruginosa* single species biofilms resulted in the significant induction of *ampC*, a beta lactamase precursor. Differential regulation of *ampC* is linked to the natural resistance of *P. aeruginosa* to this class of antibiotic (Khaledi *et al.* 2016). Imipenem, another carbapenem use to treat *P. aeruginosa* infections, also induces the expression of *ampC* (Bagge *et al.* 2004), highlighting *ampC* induction as a conserved response to carbapenems. In addition to *ampC*, the outer membrane porin, OprD, has also been associated with meropenem resistance. OprD facilitates entry of carbapenems into bacterial cells, and is therefore frequently downregulated in meropenem-resistant strains (Khaledi *et al.* 2016). In our study, *oprD* was not differentially regulated in response to meropenem treatment. However, there is often a poor correlation between *oprD* mRNA levels and protein levels (Khaledi *et al.* 2016). Therefore, it is possible that OprD protein levels are downregulated in our biofilms to reduce meropenem uptake.

Interestingly, treatment of *P. aeruginosa* biofilms with either meropenem or imipenem, results in the significant induction of biofilm associated genes (Bagge *et al.* 2004). For example, in response to meropenem extracellular polysaccharide biosynthetic genes like *pslB*, *pslD*, *pslE* and *pslF* were significantly upregulated. The induction of these polysaccharide genes results in enhanced intracellular and cell-substrate interactions, and the expression of subsets of these genes are commonly enhanced in biofilm forming clinical isolates. Psl is thought to enhance the tolerance of *P. aeruginosa* biofilms to a range of antimicrobial agents including colistin, tobramycin and ciprofloxacin (Billings *et al.* 2013).

In response to meropenem, genes involved in the Type 6 secretion system (T6SS) were also significantly upregulated including *ppkA*, *pppA*, *clpV1 icmF1*, *vgrG1* and *hcp1*. The structure of the T6SS bears similarities to the cell-puncturing needle of bacteriophage viruses; the roles of the T6SS in *P. aeruginosa* include virulence within hosts, delivery of toxins to neighbouring microbes that are competing for resources, and biofilm formation (Chen *et al.* 2015, Chen *et al.* 2020). Although there has been no previous research linking antibiotic treatment to upregulation of T6SS activity in *P. aeruginosa*, the T6SS has been implicated in drug resistance in *A. baumannii* and *K. pneumoniae* (Liu *et al.* 2017, Wang *et al.* 2018). However, in *P. aeruginosa*, the T6SS is upregulated during competition with other bacteria as a result

of kin cell lysis (LeRoux *et al.* 2015). Therefore, it is possible that the observed increase in transcription of components of the T6SS is a result of meropenem induced cell lysis.

During growth in dual species biofilms, *P. aeruginosa* upregulates genes required for quorum sensing, with all genes from the *pqsABCDE* operon being significantly upregulated. The *pqs* operon encodes several enzymes required for production of the *Pseudomonas* quinolone signal (PQS) (Higgins *et al.* 2018). *C. albicans* dependent regulation of PQS production is complex. The fungal quorum sensing molecule, farnesol, inhibits PQS production in planktonic interactions through modulation of PqsR dependent transcription of the *pqs* operon (Cugini *et al.* 2007). However, in *lasR* deficient *P. aeruginosa* strains, *C. albicans* restores PQS production through farnesol induced ROS dependent production of C4-HSL (Cugini *et al.* 2010). Increased C4-HSL results in the induction of pqsH and therefore restores PQS and phenazine production (Cugini *et al.* 2010). However, PQS is also induced under iron limiting conditions (Oglesby *et al.* 2008), resulting in increased biosynthesis of phenazines and siderophores, which both scavenge iron from the environment.

Phenazines are redox-active molecules that affect the redox balance of cells, the uptake of metabolites, gene expression, and have also been shown to enhance *P. aeruginosa* tolerance to the antibiotic, ciprofloxacin (Schiessl *et al.* 2019). Phenazines are also able to facilitate electron transfer within biofilms (Saunders *et al.* 2020). Pathways associated with the biosynthesis of these molecules were the most significantly upregulated biological process in our dual species biofilms, and in other studies highlighting their importance in interkingdom interactions.

Our transcriptional analysis identified several RND and ABC transporters to the differentially regulated in dual species biofilms in the presence of meropenem. little is known about the role of ABC transporters in *P. aeruginosa* drug resistance (Hulen *et al.* 2020), it is clear that the presence of *C. albicans* alters the expression of several efflux pumps, which may contribute to carbapenem tolerance in dual species biofilms. The *P. aeruginosa* genome encodes for at least 12 RND efflux pumps (Scoffone *et al.* 2021), three of which (MexAB-OprM, MexXY-OprM and MexCD-OprJ) have been linked to meropenem resistance (Fusté *et al.* 2013, Hassuna *et al.* 2020). MexC was induced in dual species biofilms, in response to meropenem treatment, suggesting that *P. aeruginosa* may increase efflux through MexCD-OprJ. Phenazines and their derivatives have been shown to directly regulate the expression of several efflux pumps including *mexG* (Dietrich *et al.* 2006, Sakhtah *et al.* 2016). Therefore, increased synthesis of phenazines in these biofilms could alter drug efflux and increase the tolerance of *P. aeruginosa* to meropenem. In agreement with this, enhanced production of the phenazine,

pyocyanin, promotes resistance to the beta-lactam class of antibiotics through reduced drug influx (Zhao *et al.* 2022).

Other differences between the transcriptional profiles for single and dual species biofilms in response to meropenem include genes involved in biofilm formation (*pslH*, *psll* and *algR*) and outer membrane vesicles (OMVs). AlgR is a transcriptional regulator of genes involved in alginate production (Deretic *et al.* 1989). Therefore, in addition to enhanced extracellular polysaccharide production through Psl biosynthesis, dual species biofilms will also contain increased levels of alginate. Increased alginate production is known to enhance biofilm production (Bagge *et al.* 2004), but has not been directly linked to antibiotic resistance. OMVs play important roles in biofilms including the secretion of PQS, extracellular DNA and beta-lactamase (Bomberger *et al.* 2009, Florez *et al.* 2017). Therefore, enhance OMV production in the presence of *C. albicans* may function to increase the composition and complexity of the extracellular matrix, which together with enhanced beta-lactamase secretion would increase the tolerance of *P. aeruginosa* to meropenem.

In summary, in response to meropenem *P. aeruginosa* biofilms upregulate *ampC* and biofilm formation to provide protection from meropenem. Although, dual species biofilms exhibit enhanced tolerance to meropenem, the *P. aeruginosa* transcriptional responses from dual species biofilms exposed to meropenem were similar to single species biofilms treated with meropenem, suggesting that the presence of *C. albicans* in dual species biofilms may limit the diffusion, or sequester the antibiotic leading to increased bacterial tolerance.

Conflicts of Interest

The author(s) declare that there are no conflicts of interest.

Funding information

Work in the Hall Lab is supported by an MRC Career Development Award (MR/L00903X/1) and the BBSRC (BB/R00966X/1, BB/R00966X/2). Work in the Blair Lab is support via a David Phillips Fellowship to J.M.A.B. (BB/M02623X/1). F.A. is supported by the Wellcome Trust Antimicrobials and Antimicrobial Resistance (AAMR) doctoral training programme (108876/Z/15/Z).

Author contributions

RAH and JB conceived and designed the experiments, FA performed the experiments and analysed the data. RAH wrote the manuscript.

References

Alam, F., D. Catlow, A. Di Maio, J. M. A. Blair and R. A. Hall (2020). "*Candida albicans* enhances meropenem tolerance of *Pseudomonas aeruginosa* in a dual-species biofilm." J Antimicrob Chemother 75: 925-935.

Alhede, M., T. Bjarnsholt, P. Jensen, R. K. Phipps, C. Moser, L. Christophersen, L. D. Christensen, M. van Gennip, M. Parsek, N. Høiby*, et al.* (2009). "*Pseudomonas aeruginosa* recognizes and responds aggressively to the presence of polymorphonuclear leukocytes." Microbiology (Reading) 155: 3500-3508.

Bagge, N., M. Schuster, M. Hentzer, O. Ciofu, M. Givskov, E. P. Greenberg and N. Høiby (2004). "*Pseudomonas aeruginosa* biofilms exposed to imipenem exhibit changes in global gene expression and beta-lactamase and alginate production." Antimicrobial Agents and Chemotherapy 48: 1175-1187.

Billings, N., M. Millan, M. Caldara, R. Rusconi, Y. Tarasova, R. Stocker and K. Ribbeck (2013). "The extracellular matrix Component Psl provides fast-acting antibiotic defense in Pseudomonas aeruginosa biofilms." PLoS Pathog 9: e1003526.

Bjarnsholt, T., P. Jensen, M. J. Fiandaca, J. Pedersen, C. R. Hansen, C. B. Andersen, T. Pressler, M. Givskov and N. Høiby (2009). "*Pseudomonas aeruginosa* biofilms in the respiratory tract of cystic fibrosis patients." Pediatr Pulmonol 44: 547-558.

Bomberger, J. M., D. P. Maceachran, B. A. Coutermarsh, S. Ye, G. A. O'Toole and B. A. Stanton (2009). "Long-distance delivery of bacterial virulence factors by *Pseudomonas aeruginosa* outer membrane vesicles." PLoS pathogens 5: e1000382-e1000382.

Brand, A., J. D. Barnes, K. S. Mackenzie, F. C. Odds and N. A. R. Gow (2008). Cell wall glycans and soluble factors determine the interactions between the hyphae of *Candida albicans* and *Pseudomonas aeruginosa*.

Chatterjee, M., C. P. Anju, L. Biswas, V. Anil Kumar, C. Gopi Mohan and R. Biswas (2016). "Antibiotic resistance in *Pseudomonas aeruginosa* and alternative therapeutic options." Int J Med Microbiol 306: 48-58.

Chen, L., Y. Zou, A. A. Kronfl and Y. Wu (2020). "Type VI secretion system of *Pseudomonas aeruginosa* is associated with biofilm formation but not environmental adaptation." Microbiologyopen 9: e991.

Chen, L., Y. Zou, P. She and Y. Wu (2015). "Composition, function, and regulation of T6SS in *Pseudomonas aeruginosa*." Microbiol Res 172: 19-25.

Choi, D.-S., D.-K. Kim, S. J. Choi, J. Lee, J.-P. Choi, S. Rho, S.-H. Park, Y.-K. Kim, D. Hwang and Y. S. Gho (2011). "Proteomic analysis of outer membrane vesicles derived from *Pseudomonas aeruginosa*." PROTEOMICS 11: 3424-3429.

Cugini, C., M. W. Calfee, J. M. Farrow, 3rd, D. K. Morales, E. C. Pesci and D. A. Hogan (2007). "Farnesol, a common sesquiterpene, inhibits PQS production in *Pseudomonas aeruginosa*." Mol Microbiol 65: 896-906.

Cugini, C., D. K. Morales and D. A. Hogan (2010). "*Candida albicans*-produced farnesol stimulates *Pseudomonas* quinolone signal production in LasR-defective *Pseudomonas aeruginosa* strains." Microbiology (Reading, England) 156: 3096-3107.

Deretic, V., R. Dikshit, W. M. Konyecsni, A. M. Chakrabarty and T. K. Misra (1989). "The *algR* gene, which regulates mucoidy in *Pseudomonas aeruginosa*, belongs to a class of environmentally responsive genes." J Bacteriol 171: 1278-1283.

Dietrich, L. E. P., A. Price-Whelan, A. Petersen, M. Whiteley and D. K. Newman (2006). "The phenazine pyocyanin is a terminal signalling factor in the quorum sensing network of *Pseudomonas aeruginosa*." Molecular Microbiology 61: 1308-1321.

Doern, G. V. and B. Brogden-Torres (1992). "Optimum use of selective plated media in primary processing of respiratory tract specimens from patients with Cystic Fibrosis." Journal Clinical Micorbiology 30: 2740-2742.

Fazli, M., T. Bjarnsholt, K. Kirketerp-Møller, B. Jørgensen, A. S. Andersen, K. A. Krogfelt, M. Givskov and T. Tolker-Nielsen (2009). "Nonrandom distribution of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in chronic wounds." J Clin Microbiol 47: 4084-4089.

Florez, C., J. E. Raab, A. C. Cooke and J. W. Schertzer (2017). "Membrane Distribution of the Pseudomonas Quinolone Signal Modulates Outer Membrane Vesicle Production in *Pseudomonas aeruginosa*." mBio 8: e01034-01017.

Fourie, R. and C. H. Pohl (2019). "Beyond Antagonism: The Interaction Between *Candida* Species and *Pseudomonas aeruginosa*." Journal of fungi (Basel, Switzerland) 5: 34.

Fusté, E., L. López-Jiménez, C. Segura, E. Gainza, T. Vinuesa and M. Viñas (2013). "Carbapenem-resistance mechanisms of multidrug-resistant *Pseudomonas aeruginosa*." J Med Microbiol 62: 1317-1325.

Guilhen, C., N. Charbonnel, N. Parisot, N. Gueguen, A. Iltis, C. Forestier and D. Balestrino (2016). "Transcriptional profiling of *Klebsiella pneumoniae* defines signatures for planktonic, sessile and biofilm-dispersed cells." BMC Genomics 17: 237.

Harriott, M. M. and M. C. Noverr (2009). "*Candida albicans* and *Staphylococcus aureus* form polymicrobial biofilms: effects on antimicrobial resistance." Antimicrob Agents Chemother 53: 3914-3922.

Hassuna, N. A., M. K. Darwish, M. Sayed and R. A. Ibrahem (2020). "Molecular Epidemiology and Mechanisms of High-Level Resistance to Meropenem and Imipenem in *Pseudomonas aeruginosa*." Infect Drug Resist 13: 285-293.

Higgins, S., S. Heeb, G. Rampioni, M. P. Fletcher, P. Williams and M. Cámara (2018). "Differential Regulation of the Phenazine Biosynthetic Operons by Quorum Sensing in *Pseudomonas aeruginosa* PAO1-N." Front Cell Infect Microbiol 8: 252.

Hogan, D. A. and R. Kolter (2002). "*Pseudomonas-Candida* interactions: an ecological role for virulence factors." Science 296: 2229-2232.

Hulen, C., P.-J. Racine, S. Chevalier, M. Feuilloley and N.-E. Lomri (2020). "Identification of the PA1113 Gene Product as an ABC Transporter Involved in the Uptake of Carbenicillin in *Pseudomonas aeruginosa* PAO1." Antibiotics (Basel, Switzerland) 9: 596.

Khaledi, A., M. Schniederjans, S. Pohl, R. Rainer, U. Bodenhofer, B. Xia, F. Klawonn, S. Bruchmann, M. Preusse, D. Eckweiler*, et al.* (2016). "Transcriptome Profiling of Antimicrobial Resistance in *Pseudomonas aeruginosa*." Antimicrob Agents Chemother 60: 4722-4733.

Kong, E. F., C. Tsui, S. Kucharíková, D. Andes, P. Van Dijck and M. A. Jabra-Rizk (2016). "Commensal Protection of *Staphylococcus aureus* against Antimicrobials by *Candida albicans* Biofilm Matrix." mBio 7.

LeRoux, M., R. L. Kirkpatrick, E. I. Montauti, B. Q. Tran, S. B. Peterson, B. N. Harding, J. C. Whitney, A. B. Russell, B. Traxler, Y. A. Goo*, et al.* (2015). "Kin cell lysis is a danger signal that activates antibacterial pathways of *Pseudomonas aeruginosa*." Elife 4.

Liu, L., M. Ye, X. Li, J. Li, Z. Deng, Y. F. Yao and H. Y. Ou (2017). "Identification and Characterization of an Antibacterial Type VI Secretion System in the Carbapenem-Resistant Strain *Klebsiella pneumoniae* HS11286." Front Cell Infect Microbiol 7: 442.

Lyczak, J. B., C. L. Cannon and G. B. Pier (2002). "Lung infections associated with Cystic Fibrosis." Clin Microbiol Rev 15: 194-222.

Mah, T. F. and G. A. O'Toole (2001). "Mechanisms of biofilm resistance to antimicrobial agents." Trends Microbiol 9: 34-39.

Mitchell, K. F., R. Zarnowski, H. Sanchez, J. A. Edward, E. L. Reinicke, J. E. Nett, A. P. Mitchell and D. R. Andes (2015). "Community participation in biofilm matrix assembly and function." Proc Natl Acad Sci U S A 112: 4092-4097.

Nett, J., L. Lincoln, K. Marchillo, R. Massey, K. Holoyda, B. Hoff, M. VanHandel and D. Andes (2007). "Putative Role of beta-1,3 Glucans in *Candida albicans* Biofilm Resistance." Antimicrobial Agents and Chemotherapy 51: 510-520.

Oglesby, A. G., J. M. Farrow, 3rd, J. H. Lee, A. P. Tomaras, E. P. Greenberg, E. C. Pesci and M. L. Vasil (2008). "The influence of iron on *Pseudomonas aeruginosa* physiology: a regulatory link between iron and quorum sensing." J Biol Chem 283: 15558-15567.

Ramakrishnan, M., S. Putli Bai and M. Babu (2016). "Study on biofilm formation in burn wound infection in a pediatric hospital in Chennai, India." Ann Burns Fire Disasters 29: 276-280.

Sakhtah, H., L. Koyama, Y. Zhang, D. K. Morales, B. L. Fields, A. Price-Whelan, D. A. Hogan, K. Shepard and L. E. Dietrich (2016). "The *Pseudomonas aeruginosa* efflux pump MexGHI-OpmD transports a natural phenazine that controls gene expression and biofilm development." Proc Natl Acad Sci U S A 113: E3538-3547.

Saunders, S. H., E. C. M. Tse, M. D. Yates, F. J. Otero, S. A. Trammell, E. D. A. Stemp, J. K. Barton, L. M. Tender and D. K. Newman (2020). "Extracellular DNA Promotes Efficient Extracellular Electron Transfer by Pyocyanin in *Pseudomonas aeruginosa* Biofilms." Cell 182: 919-932.e919.

Schiessl, K. T., F. Hu, J. Jo, S. Z. Nazia, B. Wang, A. Price-Whelan, W. Min and L. E. P. Dietrich (2019). "Phenazine production promotes antibiotic tolerance and metabolic heterogeneity in *Pseudomonas aeruginosa* biofilms." Nat Commun 10: 762.

Scoffone, V. C., G. Trespidi, G. Barbieri, S. Irudal, E. Perrin and S. Buroni (2021). "Role of RND Efflux Pumps in Drug Resistance of Cystic Fibrosis Pathogens." Antibiotics 10: 863.

Wang, J., Z. Zhou, F. He, Z. Ruan, Y. Jiang, X. Hua and Y. Yu (2018). "The role of the type VI secretion system *vgrG* gene in the virulence and antimicrobial resistance of *Acinetobacter baumannii* ATCC 19606." PLoS One 13: e0192288.

Xie, C., X. Mao, J. Huang, Y. Ding, J. Wu, S. Dong, L. Kong, G. Gao, C. Y. Li and L. Wei (2011). "KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases." Nucleic Acids Res 39: 316-322.

Zhao, X., Y. Jin, F. Bai, Z. Cheng, W. Wu and X. Pan (2022). "Mutation of *PA4292* in *Pseudomonas aeruginosa* Increases beta-Lactam Resistance through Upregulating Pyocyanin Production." Antimicrobial Agents and Chemotherapy 66: e00421-00422.

Wang, J., Z. Zhou, F. He, Z. Ruan, Y. Jiang, X. Hua and Y. Yu (2018). "The role of the type VI secretion system *vgrG* gene in the virulence and antimicrobial resistance of *Acinetobacter baumannii* ATCC 19606." PLoS One 13: e0192288.

Zhao, X., Y. Jin, F. Bai, Z. Cheng, W. Wu and X. Pan (2022). "Mutation of *PA4292* in *Pseudomonas aeruginosa* Increases beta-Lactam Resistance through Upregulating Pyocyanin Production." Antimicrobial Agents and Chemotherapy 0: e00421-00422.

517 **Table 1. Differential expression analysis carried out by DESeq2**

Comparison	Abbreviation	Output file
Untreated *P. aeruginosa* mono-species biofilm vs *P. aeruginosa* mono-species treated with_5 µg/mL meropenem	PA_0M vs PA_5M	P_aeruginosa_expression_ PA_0M_vs_PA_5M_.xlsx
Untreated *P. aeruginosa* mono-species biofilm vs dual species biofilms	PA_0M vs PACA_0M	P_aeruginosa_expression_ PA_0M_vs_PACA_0M.xlsx
Untreated *P. aeruginosa* mono-species biofilms vs dual species biofilms treated with 5 µg/mL meropenem	PACA_0M vs PACA 5M	P_aeruginosa_expression_ PACA_0M_vs_PACA_5M.xlsx

518

519

520 **Table 2. Top twenty differentially regulated genes in *P. aeruginosa* untreated single**
521 **biofilms vs single species biofilms treated with 5 µg/mL meropenem.**

	Gene ID	log2 Fold Change	p-value	padj
Upregulated	*ampC*	5.745634677	3.54E-46	1.95E-42
	PA4111	3.141752798	2.08E-25	1.27E-22
	hcp1	2.299594301	9.57E-20	2.64E-17
	PA0126	2.010278388	4.77E-15	6.75E-13
	clpV1	1.832477469	5.89E-09	3.65E-07
	PA4280.2	1.825681731	0.0007200374797	0.008213485384
	PA0084	1.81927681	9.59E-07	3.60E-05
	PA0083	1.812545239	2.64E-06	8.69E-05
	PA4690.2	1.811643426	0.0006939310453	0.00798165271
	PA5369.2	1.811071763	0.0007048977579	0.008057433792
	PA0668.4	1.770903731	0.0005529877155	0.006709989401
	PA0070	1.759966355	2.86E-06	9.28E-05
	PA0277	1.706435238	2.22E-11	2.04E-09
	vgrG1	1.692796239	4.23E-08	2.12E-06
	PA0466	1.68245735	3.45E-06	0.0001075909918
	PA0089	1.645610422	8.37E-08	3.89E-06
	PA0050	1.596317219	3.73E-05	0.0007623159776
	rplX	1.577791178	0.003955658099	0.03020634629

	rpsQ	1.514302023	0.003135531829	0.0252719288
	rplN	1.482472974	0.006174262969	0.04190492586
Downregulated	*PA0627*	-2.563807744	7.94E-27	6.26E-24
	PA0629	-2.554150934	7.27E-33	1.34E-29
	PA0641	-2.55319332	1.46E-27	1.34E-24
	PA0628	-2.534582315	1.07E-31	1.47E-28
	PA0638	-2.46361566	3.36E-33	9.27E-30
	PA0636	-2.444427878	2.44E-22	1.04E-19
	PA0635	-2.415846681	1.41E-22	6.50E-20
	gfnR	-2.353411699	6.68E-14	8.58E-12
	PA0630	-2.310960008	2.67E-21	8.68E-19
	PA0639	-2.28619203	5.70E-25	2.86E-22
	PA3638	-2.285364587	4.83E-11	4.17E-09
	PA0634	-2.272274644	9.09E-19	2.09E-16
	PA1168	-2.27006418	2.26E-06	7.65E-05
	PA0637	-2.231450235	9.63E-22	3.80E-19
	PA0616	-2.222013132	1.13E-25	7.79E-23
	PA0625	-2.213888097	1.84E-21	6.78E-19
	ptrB	-2.203953211	6.10E-17	1.05E-14
	PA0631	-2.195277667	2.16E-20	6.63E-18
	PA0613	-2.187796631	4.07E-18	8.32E-16
	PA0618	-2.143020354	3.73E-18	7.91E-16

Table 3. Top twenty differentially regulated *P. aeruginosa* genes in dual species verses single species biofilms

	Gene ID	log2 Fold Change	p-value	padj
Upregulated	*PA5384*	1.136608573	4.28E-06	0.0002779376198
	PA4097	1.000660434	3.17E-05	0.001214321657
	cdhB	0.9811955186	5.21E-06	0.0003198899691
	PA2161	0.9810583138	0.0003525619038	0.008808371695
	cdhC	0.936864827	9.18E-07	7.92E-05
	cdhA	0.9176339588	1.17E-09	4.57E-07
	ssrS	0.9064141369	8.59E-13	6.76E-10
	PA0698	0.9014266847	1.42E-05	0.0006654679167

	PA2349	0.8950640392	2.59E-05	0.001067775173
	PA2181	0.8814108328	9.08E-08	1.36E-05
	PA2090	0.8800287264	1.03E-07	1.50E-05
	PA2324	0.867636224	1.23E-05	0.0005976988392
	PA2213	0.8532908266	7.35E-16	1.35E-12
	PA2180	0.8294884121	1.24E-09	4.57E-07
	PA4088	0.8176661995	7.06E-09	1.77E-06
	czcC	0.8158365062	9.15E-08	1.36E-05
	phzC2	0.7984412293	3.58E-07	4.21E-05
	phzC1	0.788317377	3.04E-05	0.00117677973
	PA4098	0.7866314328	0.0005949449343	0.01341483781
	phzA1	0.7731714023	4.59E-06	0.0002943805348
Downregulated	*PA3572*	-0.9273548369	0.003059228691	0.0450911657
	argB	-0.8972467441	3.86E-09	1.02E-06
	rnpB	-0.8480655754	3.66E-08	5.77E-06
	PA1746	-0.7808094482	2.35E-05	0.0009898437811
	PA4351	-0.7261070956	2.78E-05	0.00110592551
	PA5303	-0.6697670409	3.36E-13	3.09E-10
	ftsY	-0.5672143027	1.04E-08	2.29E-06
	PA4276.1	-0.5616231924	1.53E-05	0.0006970521393
	PA4690.3	-0.5391253382	0.002180107776	0.03467439429
	PA1137	-0.5377222343	1.98E-11	9.95E-09
	PA3133.3	-0.5289323628	0.0003527179099	0.008808371695
	ssrA	-0.5226447106	3.43E-06	0.0002369715977
	PA3573	-0.5188361793	6.23E-05	0.002219824419
	PA4421	-0.5151637325	1.87E-11	9.95E-09
	PA1796.1	-0.4988013205	0.001465215919	0.02591835467
	PA4637	-0.4958503362	0.001596304616	0.02744549898
	PA1510	-0.4875966167	3.42E-08	5.55E-06
	PA3951	-0.4870639148	9.51E-09	2.25E-06
	PA2943	-0.4748718073	1.76E-06	0.0001407992501
	PA3277	-0.4616098795	0.0007070580094	0.01530295354

Table 4. Top twenty differentially regulated *P. aeruginosa* genes in dual species biofilms treated with 5 μg/mL meropenem vs untreated dual species biofilms.

	Gene ID	log2 Fold Change	p-value	padj
Upregulated	*ampC*	5.515387278	7.06E-51	1.95E-47
	PA4280.5	3.700797106	7.82E-06	0.0002259666295
	PA0668.1	3.67825942	8.05E-06	0.0002315796869
	PA4690.5	3.49778215	2.92E-05	0.0007129969543
	PA5369.5	3.485074044	3.01E-05	0.0007281076683
	PA4111	2.963866877	8.30E-18	1.99E-15
	PA4690.2	2.605990986	0.003138749477	0.03261549984
	PA5369.2	2.604799667	0.003142239391	0.03261549984
	PA4280.2	2.596702641	0.003658501903	0.03633497754
	PA0668.4	2.58797494	0.003177734857	0.03286039678
	hcp1	2.353244054	1.14E-22	9.02E-20
	PA0466	1.908730924	1.39E-11	1.30E-09
	pchG	1.899939854	1.07E-08	6.09E-07
	PA0126	1.883859984	5.23E-13	6.57E-11
	PA0050	1.820434031	2.26E-06	7.78E-05
	clpV1	1.75537326	1.53E-11	1.37E-09
	vgrG1	1.730154359	3.27E-12	3.37E-10
	PA4222	1.708263864	2.26E-12	2.45E-10
	PA0563	1.691305244	1.68E-11	1.47E-09
	PA0086	1.677560955	4.89E-10	3.46E-08
Downregulated	*PA0627*	-2.524464134	1.52E-15	2.79E-13
	gfnR	-2.418533396	8.72E-23	8.02E-20
	PA0641	-2.404904976	2.04E-20	7.49E-18
	PA0638	-2.364308134	4.30E-21	1.98E-18
	PA0628	-2.298437047	1.97E-20	7.49E-18
	PA0635	-2.263949045	1.95E-16	3.84E-14
	PA0629	-2.260720569	5.93E-19	1.92E-16
	PA0639	-2.220599378	2.18E-21	1.21E-18
	PA0636	-2.211742782	4.17E-18	1.15E-15
	PA0625	-2.099597686	3.91E-17	8.30E-15
	PA0616	-2.045775386	2.09E-15	3.72E-13
	PA3638	-2.041401724	2.33E-18	7.14E-16
	PA0634	-1.982462548	1.30E-17	3.00E-15
	PA1168	-1.971155931	1.76E-14	2.56E-12

	PA0631	-1.92975403	4.75E-15	7.95E-13
	PA0619	-1.919212071	1.02E-13	1.31E-11
	PA3631	-1.891142906	4.13E-19	1.43E-16
	PA0626	-1.882097335	5.95E-17	1.22E-14
	PA0630	-1.820934828	4.77E-11	3.71E-09
	PA0637	-1.809932956	1.14E-14	1.75E-12

Figure 1. Summary of transcriptional responses of *P. aeruginosa* biofilm cells to *C. albicans* and to meropenem treatment. Numbers of significantly differentially expressed *P. aeruginosa* transcripts (Padj ≤ 0.05) in 1) *P. aeruginosa* single-species biofilms treated with 5 µg/mL meropenem; 2) dual species untreated biofilms; 3) dual species biofilms treated with 5 µg/mL meropenem; 4) single vs dual species biofilms treated with 5 µg/mL meropenem. PA: *P. aeruginosa*; CA: *C. albicans*. Red: numbers of significantly upregulated transcripts; Blue: numbers of significantly downregulated transcripts.

Figure 2. *C. albicans* enhances the tolerance of *P. aeruginosa* to meropenem after short exposures. Preformed 24 hr biofilms were incubated in Mueller Hinton broth containing 0 or 5 µg/mL meropenem, for 4 hr and viable *P. aeruginosa* counts quantified. Data are the mean ± the SEM from 4 biological replicates. Data were analysed using 2-way ANOVA and Holm-Sidak's multiple comparisons test (ns not significant; * $P < 0.05$).

Figure 3. Meropenem treatment increases the expression of genes required for biofilm formation and iron homeostasis. **A)** GO term enrichment analysis and **B)** KEGG analysis of genes significantly differentially regulated in *P. aeruginosa* single species biofilms by meropenem. Bars in red represent pathways/processes that were significantly upregulated, while bars in blue represent pathways/processes that were significantly downregulated. Data were analysed using Fisher's exact test and Benjamini and Hochberg method for FDR correction (* FDR ≤ 0.05).

Figure 4. *P. aeruginosa* upregulates genes that provide a competitive fitness advantage during growth in dual species biofilms. A) GO term enrichment analysis and **B)** KEGG analysis of *P. aeruginosa* genes significantly differentially regulated in dual species biofilms. Bars in red represent pathways/processes that were significantly upregulated, while bars in blue represent pathways/processes that were significantly downregulated. Data were

analysed using Fisher's exact test and Benjamini and Hochberg method for FDR correction (* FDR ≤ 0.05).

Figure 5. Dual species biofilms do not perturb the *P. aeruginosa* transcriptional response to meropenem. A) GO term enrichment analysis and **B)** KEGG analysis of *P. aeruginosa* genes significantly differentially regulated in dual species biofilms in response to meropenem treatment. Bars in red represent pathways/processes that were significantly upregulated, while bars in blue represent pathways/processes that were significantly downregulated. Data were analysed using Fisher's exact test and Benjamini and Hochberg method for FDR correction (* FDR ≤ 0.05).

Figure 6. Identification of overlapping differentially expressed genes in *P. aeruginosa* mono and dual species biofilms in response to meropenem. Numbers of significantly upregulated (a) and downregulated (b) genes (Padj ≤ 0.05), in response to treatment with 5 µg/mL meropenem, are compared between single- and dual-species biofilms (PA *P. aeruginosa*; CA *C. albicans*). Genes of interest, unique to the dual-species condition, are listed on the right-hand side.

Supplemental Figure 1. PCA plots **A)** single species *P. aeruginosa* in response to meropenem. **B)** Dual species biofilms in the absence of meropenem. **C)** Dual species biofilms in the presence of meropenem.

Supplemental Figure 2. Transcriptional profiling identifies genes differential regulated in single and dual species biofilms in response to meropenem. Volcano plots identifying significantly differentially regulated genes in **A)** single species *P. aeruginosa* in response to meropenem. **B)** Dual species biofilms in the absence of meropenem. **C)** Dual species biofilms in the presence of meropenem.